Exploring fragment space

de Kloe, G.E.

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Aim and Outline

AIM OF THIS THESIS

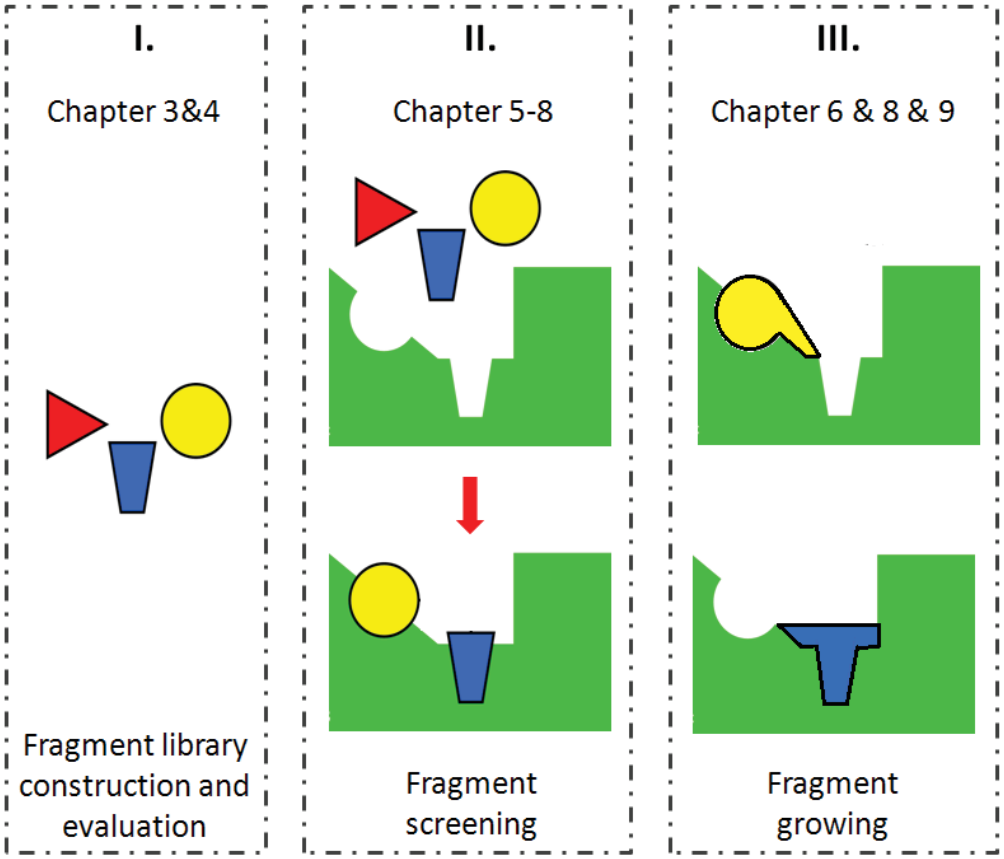
As described in the review in Chapter 1, the first fragment-based medicinal chemistry study was published in 1997. At the time the research covered in this thesis started, end of 2006, various industries had started FBDD programs, following pioneer Abbott. A couple of interesting studies had appeared concerning theoretical questions underlying the fragments dogma. Just at the start of this project, a retrospective study on fragment growing was published by Abbott. The observation of a linear relationship between molecular weight and binding affinity for the 18 deconstructed lead compounds that were analyzed, led quickly to the 'rule' of keeping the ligand efficiency (LE) constant during fragment growth.

The mentioned retrospective study, limited to enzymes, triggered us to start our FBDD study on the nicotinic acetylcholine receptors (nAChRs) with the deconstruction of known ligands. The resulting reference set of fragments, covering a range of molecular weights, was ideal to validate screening methods that were required. The knowledge with respect to LEs of the fragmented set could be used to facilitate fragment hit prioritization and exploration. To enable fragment screening, the water-soluble acetylcholine binding protein (AChBP) is used as a model protein for the nAChRs.

The aim of this study can be summarized in three points:

1. The construction and analysis of a suitable fragment library
2. The set up and evaluation of biochemical and biophysical fragment screening approaches
3. The optimization of hits by growing and/or linking fragments

OUTLINE OF THIS THESIS



I. FRAGMENT LIBRARY CONSTRUCTION AND EVALUATION

The project started with the construction of a fragment library. In **Chapter 3** fragment library design, as reported in scientific literature, is reviewed. In **Chapter 4**, the construction and analysis of our fragment library is described. A diverse library of 1010 fragments was obtained by applying filters to the in-house compound database, followed by diversity analyses. These analyses leaded to the selection of a diverse subset of fragments, as well as the purchase of novel scaffolds. The 1010 fragments have been screened against a panel of targets; the resulting hits have been analyzed with respect to physico-chemical properties and target affinity profiles.

II. FRAGMENT SCREENING

Chapter 5 describes the setup of an SPR Biosensor assay for fragment screening on AChBP. Kinetic data is discussed as well as the ability to measure conformational changes. In **Chapter 6** the assay was further validated with a reference set of fragments of diverse molecular weights and affinities. This reference set, obtained by deconstruction of a high affinity ligand for the α7 nAChR, led to the identifications of ligand efficiency hot spots in AChBP and nAChRs.

A novel biochemical assay was developed for AChBP, making use of a ligand with enhanced fluorescent properties upon binding (**Chapter 7**). This assay was used in an online setting, with the possibility of mixture screening by LC separation followed by MS identification. The online biochemical assay was also perfectly suited for fragment library screening, hit validation and ranking, and rapid hit exploration by combinatorial chemistry at 96-well format, as described in **Chapter 8**.

III. FRAGMENT HIT EXPLORATION

The final **Chapter 9** describes ligand- and structure-based optimization efforts on a hit fragment. After a 50-fold increase in affinity by analogue screening, structure-based design led to the definition of three optimization vectors. Subsequent synthesis and pharmacological results are described.